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SPATIAL ECOLOGY OF THE WESTERN MASSASAUGA (*SISTRURUS TERGEMINUS*) IN A LARGE

INTERIOR WETLAND

being

A Thesis Presented to the Graduate Faculty

of the Fort Hays State University in

Partial Fulfillment of the Requirements for

the Degree of Master of Science

by

Joshua Mead

B.S., University of Nebraska–Lincoln

Date Approved

Major Professor

Approved

Chair, Graduate Council

This thesis for

The Master of Science Degree

By

Joshua Mead

has been approved by

Chair, Supervisory Committee

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ABSTRACT

Insight into the spatial ecology of a population of animals provides information valuable to the management and conservation of a species. Reptiles are facing global declines, with 1 in 5 species currently threatened with extinction. For cryptic taxa such as snakes, radio-telemetry allows for individuals to be reliably located on a consistent basis. I used radio-telemetry to investigate the spatial ecology of the Western Massasauga (*Sistrurus tergeminus*) at Cheyenne Bottoms in Barton County, Kansas. Eighteen individuals (12 male and 6 female) were implanted with very high frequency (VHF) radio transmitters during 2016-2017 and tracked twice weekly from July to November in 2016 and April to October in 2017. Home range estimates were calculated for 15 individuals with 30 or more unique locations using minimum convex polygons (MCP) and kernel density estimators (KDE). Average home range estimates for these individuals using 100% MCP were 12.90 ha (0.37-60.99 ha) and 1.41 ha (0.07-5.12 ha) for 95% KDE. Core use area estimates using 50% MCP averaged 2.80 ha (0.05-13.48 ha) and 0.06 (0.001-0.21 ha) for 50% KDE. Male estimates were larger than female estimates across all estimators. Estimates for *S. tergeminus* on the constrained landscape of the Cheyenne Bottoms Wildlife Area (CBWA) were smaller than estimates on the more natural and less manipulated Cheyenne Bottoms Preserve, although the sample size was much larger on the CBWA. This study provides important insight into the biology of *S. tergeminus* and is information that can be used to help conserve this species across its range.

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PREFACE

This thesis is written in the style of the Society for the Study of Amphibians and Reptiles. All handling and surgical procedures in this study were done in accordance with the joint herpetological society Guidelines for Use of Live Amphibians and Reptiles in Field Research. These methods were approved by the Institutional Animal Care and Use Committee of Fort Hays State University (IACUC protocol #16-0003) and conducted under State of Kansas Scientific, Education, or Exhibition Wildlife Permits SC-080-2016 and SC-092-2017.

INTRODUCTION

Global threats to biodiversity continue to increase and are the result of anthropogenic causes, including habitat destruction and over-exploitation of natural resources (Butchart et al., 2010; Maxwell et al., 2016). These threats are causing the loss of biodiversity to occur at increased rates (Skole and Tucker, 1993; Brooks et al., 2002). In spite of pressure by the political leaders of the world to slow this trend, these rates have not decreased (Butchart et al., 2010). It is beneficial to conserve biodiversity as animals serve many ecosystem and economic functions including crop pollination, soil erosion control, and pest control, in addition to their intrinsic value (Myers, 1996; Cleveland et al., 2006). Wildlife also serves as an important source of protein in developing countries. The people in these areas are likely to suffer as biodiversity is lost (Díaz et al., 2006).

Currently, 1 in 5 of the over 9,000 species of extant reptiles are threatened with extinction (Uetz, 2010; Böhm et al., 2013). Reptiles suffer from the same stressors as all animals. However, due to their lower vagility compared to birds and many mammals, reptiles can suffer significant mortality and genetic diversity loss from small scale habitat fragmentation (Clark et al., 2010; Gerow et al., 2010). Additionally, reptiles suffer persecution from society because of beliefs, fears, and commercial exploitation (Adams et al., 1994; Zhou and Jiang, 2004; Crump, 2015). Even though perceptions of these animals vary widely, they provide important services and products to mankind. These include anti-diabetic drugs developed from the venom of the Gila Monster (*Heloderma suspectum*), geckos functioning as bioindicators of air quality, and important model

1

organisms for biological research in physiology (Read, 1998; Beaupre and Zaidan, 2012; Furman, 2012).

 If we are to prevent further declines of these animals, an understanding of their basic ecology will be needed to develop effective strategies for their conservation. However, the detailed information of snake populations needed to detect declines is difficult to acquire due to their secretive nature (Mazerolle et al., 2007; Willson et al., 2011). With the continued conversion of landscapes for urban development and intensive agriculture, these declines are forecasted to continue if conservation measures are not enacted (Bongaarts, 2009; Deng et al., 2009; Reading et al., 2010). Accordingly, understanding the spatial requirements of snakes has never been of more importance to their conservation (Robinson et al., 1992; Blouin-Demers and Weatherhead, 2002).

Snakes (Squamata: Serpentes) are a limbless group of tetrapods comprised of over 3,400 species worldwide (Brandley et al., 2008; Pough et al, 2016). Snakes serve as predators and prey in many ecosystems (Chapman and Casto, 1972; Trauth and McAllister, 1995; Holycross et al., 2002). Their role as predators has an economic value in pest control of rodents, including species known to carry infectious zoonotic diseases like hantavirus (Calisher et al., 2001).

 Rattlesnakes are a specialized group of New World vipers (Serpentes: Viperidae) represented in 2 genera (*Crotalus* and *Sistrurus*) and includes approximately 20 species in the United States (Crother et al., 2017). These snakes have a modified keratin structure at the end of their tail that generates an audible warning when the tail is oscillated, and is responsible for their colloquial name. Rattlesnakes are commonly persecuted because of fear of envenomation or a general dislike of snakes (Klauber, 1956). Nevertheless,

rattlesnakes are an important predator in many ecosystems and have direct value to humans because of pharmaceutical products derived from their venom (Phillips and Scarborough, 1997; Holycross and Mackessy, 2002; Kupferschmidt, 2013).

 The Western Massasauga (*Sistrurus tergeminus*) is a small species of rattlesnake native to the central United States and northern Mexico (Fig. 1), from Nebraska in the north to Coahulia in the south (Powell et al., 2016). Kubatko et al. (2011) elevated *S. catenatus tergeminus* to a distinct species from the Eastern Massasauga (*S. catenatus*). Currently, 2 subspecies of *S. tergeminus* are recognized, the Desert Massasauga (*S. t. edwardsii*) of Colorado, New Mexico, Arizona, Texas, and Mexico, and the Prairie Massasauga (*S. t. tergeminus*) of Nebraska, Iowa, Missouri, Kansas, Oklahoma, and Texas (Kubatko et al., 2011; Powell et al., 2016; Crother et al., 2017) (Fig. 1). In Kansas, the range of *S. t. tergeminus* includes the eastern two-thirds and southwestern quarter of the state where it inhabits a wide range of microhabitats (Collins et al., 2010). Bender (2009) documented mammals, snakes, lizards, and centipedes in the diet of *S. tergeminus* in Kansas. This species reaches its highest population densities in mesic habitats in Kansas, where it also grows to a larger size on average (Bender, 2009; Collins et al., 2010).

S. tergeminus does not have protected status in Kansas but is currently listed as threatened in Nebraska and endangered in Missouri (MDC, 2018; NGPC, 2018). However, *S. catenatus* is federally threatened and mortalities due to snake fungal disease have been documented in this congener (Allender et al., 2011; Lorch et al., 2016; USFWS, 2018). In addition, recent study suggested that weather affects the body size, reproduction, and growth of *S. catenatus* (Hileman et al., 2017). These sources of

environmental stress might affect *S. tergeminus* and it seems prudent to consider the conservation of *S. tergeminus* in Kansas before declines occur.

The spatial ecology of a species represents how organisms use the landscape, and one way to quantify an aspect of this use is through the estimation of home range (Millspaugh and Marzluff, 2001). A home range is the area used by an animal during its normal activities (Burt, 1943). This information is important to gather from healthy populations because it can be difficult to detect when population declines occur (Gibbons et al., 2000).

To better understand how specific individuals use the area, they must be frequently and consistently located (Millspaugh and Marzluff, 2001). Radio-telemetry allows specific individuals to be consistently located and is one of the only ways to obtain predictable locality information for secretive species. Very high frequency (VHF) transmitters emit a pulsed radio signal that can be detected by an antenna and receiver. Signal strength is a function of the proximity and the angle of incidence to the transmitter from the antenna. (Millspaugh and Marzluff, 2001).

I studied the spatial ecology of *S. tergeminus* at Cheyenne Bottoms in Barton County, Kansas. The objectives of this study were to identify if significant differences existed in the spatial ecology of *S. tergeminus* by sex or landscape type, and to provide estimates of home range and core use areas that might be used to conserve this species in Kansas. At my study site, one property has constricted land features that result from a series of water control structures, and the other property has a more natural landscape bisected by only few roads. I hypothesized that *S. tergeminus* on the more constricted but apparently highly productive land would have smaller home range and core use areas

than the more natural landscape. The more constrained landscape has apparently high densities of animals that are more frequently encounter by chance than seen on the more natural landscape. Several reasons for this could exist, but the concentration of land from water control structures could result in these observations. This leads to the hypothesis that this apparently resource rich area would result in smaller home ranges from a reduction in the need to move to find essential resources. I also hypothesized that males would have larger home ranges than females. Males are commonly documented to have larger home ranges in vipers, and this has commonly attributed to searching for reproductive opportunities during the breeding season (Reinert and Zappalorti, 1988; Duvall and Schuett, 1997). While studies in adjacent states have investigated the spatial ecology of *S. tergeminus* (Durbian et al., 2008; Wastell and Mackessy, 2011; Patten et al., 2016), this is the first study to investigate the spatial ecology of *S. tergeminus* in Kansas. Because the spatial requirements of snake populations are known to vary among populations, regional studies of specific populations are important and necessary if we are to make management decisions to conserve this species (Shine, 1987; Gibbons et al., 2000; Durbian et al., 2008).

MATERIALS AND METHODS

Study Site. – Cheyenne Bottoms is an approximately 16,600 ha lowland basin located in Barton County, Kansas, USA. This large interior wetland was historically sustained by surface flow from Blood and Deception creeks. Of this area, 8,036 ha are owned and managed by the Kansas Department of Wildlife, Parks and Tourism (KDWPT) as the Cheyenne Bottoms Wildlife Area (CBWA) and 3,114 ha are owned and managed by The Nature Conservancy (TNC) as the Cheyenne Bottoms Preserve (CBP). These properties and a few privately-owned adjacent parcels make up the study area.

The study area has little topographic relief and is largely void of trees except for a few scattered along road ditches. Privately-owned areas adjacent to these study sites are primarily production agriculture. The TNC property has largely been removed from agricultural production and is being restored to treeless, seasonal marshes with natural flows of sheet water (R. Penner, pers. comm.). The vegetation of this area consists of grasses and small forbs including Alkali Sacaton (*Sporobolus airoides*), Prairie Cordgrass (*Spartina pectinata*), Saltgrass (*Distichlis spicata*), and Cattails (*Typha* sp*.*) near permanent or semi-permanent water.

The KDWPT property is a modified landscape dominated by dikes and canals constructed in the 1950s to control water levels, and to divert water from the Arkansas River and Walnut Creek (Fig. 2). The center pool (Pool 1) is a 1,335 ha shallow reservoir (approximately $1 - 1.25$ m deep) and holds water most years (Fig. 3). The area of Pool 1 and a varying portion of other pools is normally not usable area for *S. tergeminus* due to the presences of standing water. The surrounding pools (Pools 2-5) have been invaded by Cattail (*Typha* sp.) and are the focus of active management by the KDWPT.

Shallow standing water in Pools 2 - 5 dissipates to peripheral marshes. The dikes that separate the pools are dominated by Reed Canarygrass (*Phalaris arundinacea*), Brome (*Bromus* sp.), and seasonally by Poison Hemlock (*Conium maculatum*). In areas of the KDWPT property that are not inundated, Saltgrass (*Distichlis spicata*) is common and often forms continuous mats.

The focus of management on CBWA is to provide habitat to support migratory flocks of waterfowl and shorebrids. Approximately 5,260 ha of the study areas are seasonally open to hunting (KDWPT, 2010). Cheyenne Bottoms is located in the central flyway of North America and is one of the most important staging areas for migratory birds in the United States (Zimmerman, 1990). In spring 1986, it was estimated that approximately 11,000 kg of blood worms (Diptera: Chironomidae) were consumed by migratory shore birds (KBS, 1987). This area is a popular birdwatching and hunting location and consistently experiences high numbers of both user groups (KDWPT, 2010). The high amount of human interest in these opportunities creates a constant treat to snakes crossing roads in these areas.

Cheyenne Bottoms also supports healthy populations of resident wildlife including *S. tergeminus*, which occur at high densities (Collins et al., 2010). In addition, *S. tergeminus* in Cheyenne Bottoms were on average larger in size compared to populations in nearby grasslands (Bender, 2009).

Surveys. – I surveyed for *S. tergeminus* via visual encounter surveys from July 2016 through May 2017. These surveys were conducted on foot through suitable habitat and in motor vehicles on roads adjacent to suitable habitat. Surveys for individuals

greater than 180g were conducted to ensure transmitter mass did not exceed 5% of the individuals total body mass (Johnson, 2000; DeGregorio et al., 2011; Clark et al, 2012).

Surgical Procedure.– Transmitters were surgically implanted at the Rohleder Veterinary Service facility (Hays, Kansas), under the supervision of Drs. Jessica Braun and James Rohleder. I used Holohil SI-2 transmitters (9 g; 33 mm x11 mm) that had an average battery life of 12 months and a potential battery lifespan range of 6-18 months (Holohil Systems Ltd., Ontario, Canada). Transmitters were sterilized in a bath of benzalkonium chloride for 1-2 hours (Hardy and Greene, 1999). Surgical procedures were adapted from Reinert and Cundall (1982) and Mader and Divers (2013). Individuals were restrained in appropriately sized plastic tubes (Midwest Tongs, Inc, Missouri, USA), that were sealed with a hose connected to an anesthesia machine. Isoflurane gas was administered at 5% until the individual reached a surgical plane of anesthesia, at which point the isoflurane gas was reduced to a maintenance dose of 2-3% for the duration of the procedure. If a surgical plane of anesthesia was not achieved within 45 minutes of exposure, ketamine was injected subcutaneously (SC) at 5 to 10 mg/kg.

During induction into a surgical plane of anesthesia, the analgesics hydromorphone (1.0 mg/kg SC) and meloxicam (0.3 mg/kg SC) were administered. Once the subject achieved a surgical plane of anesthesia, the locations of both incisions were sterilized with chlorhexidine gluconate or betadine and a surgical drape was placed over the animal. Sterile technique was used during the entire surgery. The transmitter was removed from the benzalkonium chloride bath and rinsed with sterile saline solution (B. Kingsbury, pers. comm.). I made a 3-5 cm incision between the second and third scale rows dorsal of the venter on the right side of the individual at approximately 1/3 of the

snout-vent length anterior of the cloaca. The transmitter was inserted through the ribs and into the coelomic cavity (Fig. 4).

I made a 1-cm incision approximately 22 cm anterior to the first incision and between the second and third scale rows dorsal of the venter. A sterile 1/16 in x 0.014 in copper tube was inserted subcutaneously from the anterior incision along the right side until the tube was confluent with the first incision. The antenna of the transmitter was thread through the lumen of the copper tube and then the copper tube was removed through the anterior incision. Both incisions were closed using 4/0 PDS Plus suture and sealed with Medbond tissue glue. Pure oxygen was provided for several minutes postsurgery. Mean surgery time for transmitter implantation was 26 minutes. The individual was allowed to recover in captivity for 3-5 days post-surgery and released at the capture location (DeGregorio et al., 2011).

*Radio-*telemetry.– Individuals were tracked twice a week from July to November in 2016 and from April to October in 2017 using a Wildlife Materials TRX-1000 receiver and a 3-element yagi antenna (Wildlife Materials, Inc., Illinois, USA). Visual confirmation of individuals was attempted at least once weekly. When visual confirmation was not possible or attempted, the animals were typically confirmed within a 1 m area. Tracking events were never on consecutive days. The order of individuals tracked was altered for each event to avoid locating the same individual at the same time during each event. Individual siting locations were measured using a Garmin 550t (Garmin International, Inc., Kansas, USA).

Spatial Analysis. – Home range and core use area estimates were calculated using Biotas 2.0 Software (Ecological Software Solutions LLC, Hegymagas, Hungary). All

coordinates recorded from the Garmin 550t were converted from decimal degrees to easting and northing in ArcMap 10.5 (ESRI Geographic Information Systems, California, USA) for input into Biotas.

 Minimum convex polygons (MCP) were calculated at 100% and 50% using a stationary arithmetic mean centroid. MCP is a non-statistical technique that creates the smallest possible polygon around the specified percent of the location points (Hayne, 1949). This technique is commonly used and has been recommended for estimating home ranges of herpetofauna because of its repeatability (Row and Blouin-Demers, 2006). The 100% MCP gives insight into the space an individual uses during its normal activities, or home range; while the 50% MCP gives insight into a more focused area that the animal was using during the year, or core use area (Burt, 1943; Johnson, 2000). The 100% MCP is one of the most commonly used estimators of home range for snakes (Johnson, 2000; Durbian et al., 2008; Wastell and Mackessy, 2011).

Fixed-kernel density estimates were calculated at 95% and 50% using leastsquares cross validation (LSCV) to determine a smoothing factor. Kernel density estimators (KDE) are a nonparametric statistical technique that estimates the utilization distribution, or the area of locations over a specified amount of time (Van Winkle, 1975; Worton, 1989; Seaman and Powell, 1996). The 95% KDE gives insight into home range and the 50% KDE gives insight into core use area, as previously described. Fixed KDE do not allow the smoothing factor to vary during the calculation of an estimate, are a more conservative method than adaptive KDE, and perform well when using LSCV to select a smoothing factor (Wonton, 1989; Seaman et al., 1999). LSCV is commonly used to determine a smoothing factor in herpetofaunal spatial investigations (Durbian et al.,

2008; DeGregorio et al., 2011; Wastell and Mackessy, 2011) and is the most robust for home range analysis in general (Seaman and Powell, 1996). The comparability of KDE using LSCV in studies focused on home range in herpetofauna has been questioned (Row and Blouin-Demers, 2006), however, I provided these kernel density estimates for comparison because most studies of *S. tergeminus* have reported them (Durbian et al., 2008; Wastell and Mackessy, 2011; Patten et al., 2016). Estimates were only calculated for individuals with 30 or more unique locations (Seaman et al., 1999). When individuals were tracked over both seasons, all locality points were used in these analyses.

RESULTS

Radio-telemetry.– A total of 18 adult *S. tergeminus* were implanted with VHF transmitters between July 2016 and June 2017. Body masses of these individuals ranged from $171.4 - 375.5g$. Transmitters accounted for less than 5% of total body mass $(2.40 -$ 5.25% of the total body weight of the individual) with 2 exceptions (St07 - 5.09% and St13 - 5.25%). However, the transmitter mass relative to the mass of these individuals was below the 6.5% reported in previous studies on *S. tergeminus* (Patten et al, 2016) and below the 10% maximum recommended in the Guidelines for the Use of Live Amphibians and Reptiles in Field and Laboratory Research (Beaupre et al., 2004).

In 2016, a total of 5 individuals were implanted and tracked from 25 July to 11 November. Four of these individuals survived into the 2017 field season. An additional 13 individuals were implanted in 2017 and tracked from 02 April to 26 October in 2017. Of these 18 individuals, 12 were males and 6 were females. Individuals were distributed throughout the study area; 3 individuals (2 males and 1 female) on the Cheyenne Bottoms Preserve (CBP) owned by The Nature Conservancy (TNC) and 15 individuals (10 males and 5 females) on the Cheyenne Bottoms Wildlife Area (CBWA) owned by the Kansas Department of Wildlife, Parks and Tourism (KDWPT).

Effects of Transmitters on Individuals.– No formal experiment was designed to test the effects of transmitters on the behavior of individuals. Individuals were frequently inspected for any sign of physical impairment. Anecdotal observations suggest that transmitters had minimal impacts, if any. Individuals were observed post-surgery exhibiting courtship (St03, St08, St11, St13, St14, and St15) and breeding behavior (St02). One male (St08) moved over 0.8 km in 4 days within 2 weeks post-surgery and

was discovered on top of a female that was later implanted (St14). She also gave birth to a litter. One female (St03) was implanted late in her pregnancy and gave birth. Another female (St13) also gave birth to a litter post-surgery. No signs of infection or impaired movement were observed during this study.

Home Range.– Home range estimates were calculated for 15 individuals with 30 or more unique locations (average $= 49.6$ locations, range $= 40-74$ locations). Mean home range estimates for these individuals using 100% MCP (Table 1, Fig. 5 & 6) were 12.90 ha (0.37-60.99 ha) and 1.41 ha (0.07-5.12 ha) for 95% KDE (Table 1, Fig. 7 & 8). Core use area estimates using 50% MCP were 2.80 ha (0.05-13.48 ha) and 0.06 (0.001-0.21 ha) for 50% KDE.

Mean home range size and core use area estimates were larger among males (Table 2) compared to females (Table 3). Mean home range estimates for males were 16.05 ha (0.89-60.99 ha) for 100% MCP and 1.85 ha (0.07-5.12 ha) for 95% KDE, and the mean for females was 6.60 ha (0.37-22.00 ha) for 100% MCP and 0.51 ha (0.11-1.66 ha) for 95% KDE. Mean core use area estimates for males were 3.00 ha (0.10-13.48 ha) for 50% MCP and 0.06 (0.00-0.16 ha) for 50% KDE, and females means were 2.41 ha (0.05-6.58 ha) for 50% MCP and 0.05 (0.004-0.21 ha) for 50% KDE.

Mean home range and core use areas were larger on the TNC property than in the KDWPT property. Mean home range sizes for the 3 individuals on the TNC property were 28.27 ha (5.85-60.99 ha) for 100% MCP and 2.07 ha (0.27-5.12 ha) for 95% KDE. Core use area mean estimates on the TNC property were 7.30 ha (3.69-13.48 ha) for 50% MCP and 0.06 ha (0.01-0.16 ha) for 50% KDE. Mean home range size for the 15 individuals on the KDWPT property were 9.05 ha (0.37-22.00 ha) for 100% MCP and

1.24 ha (0.07-4.13 ha) for 95% KDE. Core use area mean estimates on KDWPT property were 1.67 ha (0.05-6.58 ha) for 50% MCP and 0.05 ha (0.00-0.21 ha) for 50% KDE.

Habitat Use.–Five individuals (St01, St02, St05, St09, and St12) were observed on cattails above standing water, for various durations. Of these individuals, St09 used the cattails for the longest duration and was observed in them for 26 consecutive events from 22 June through 21 September 2017.

On 21 September 2017, St09 was observed approximately 0.25 km from last known location and positioned in cattails above standing water at the edge of a dike road (Fig. 9). He was discovered on the dike road on 26 September. Considering the distance to dry land, and the mean amount of movement observed between these events, it seems unlikely that this individual left the cattails during this time.

Movements.–Movements of individuals seemed to vary by season. Long distance movements were observed by males during the spring. These movements would extend to as much as 0.8 km between observations (3-4 days). Two males (St02 and St04) crossed open water on several occasions. St02 crossed the inlet canal south of Pool 2 and north of Pool 5 on the KDWPT property, and St04 crossed Shop Creek on the TNC property on several occasions (Fig. 2). Individuals that used cattails also crossed open water.

Hibernacula.–Four of the 5 individuals implanted in 2016 survived to burmation, the equivalent of hibernation in ectotherms, in 2016. Hibernacula for 3 individuals (St01, St02, and St03) were crayfish burrows. The burrow for the fourth individual (St04) could not be located. In 2017, 12 of the 18 individuals implanted during the 2 field seasons survived to the end of October. Only 1 of the individuals (St04) implanted in 2016

survived to the end of the 2017 active season. Five of the 6 deaths are confirmed deaths, while one is unknown and could be related to transmitter failure.

Predation.– Six of the 18 *S. tergeminus* implanted with VHF transmitters during this study died during the study (Table 4). On 14 October 2016, I recovered the transmitter of St05 and it was completely clean of any tissue and possessed small puncture marks. These puncture marks were consistent with the dentition of a mesocarnivore such as the Striped Skunk (*Mephitis mephitis*) or Mink (*Neovison vison*), both of which are common at this site. There was no sign of the carcass. The individual appeared healthy and active at the previous tracking event. A similar event was documented in the spring of 2017. The transmitter of St02 was recovered cleaned of all tissue (but no puncture marks) after 10 successful tracking events in 2017. The individual appeared healthy and active during the previous tracking event.

A 20-30 mm scar was observed on St03 on 01 June 2017. I suspect the wound resulted from a failed predation attempt. This female also appeared under-weight at this time. The condition of St03 gradually declined until it was found dead but intact on 15 June 2017. Approximately 1/3 of the body of a male (St01) was found with the transmitter present. The body was substantially decayed. The cause of death was unknown.

On 17 August 2017, a pregnant female (St14) was discovered in the coils of a Speckled Kingsnake (*Lampropeltis holbrooki*) in a rocky area adjacent to Pool 1. Upon discovery, the *L. holbrooki* retreated into an area of broken pieces cement. Two preecdysis neonates were observed within 1 m of the deceased female, though more could

have been hidden in the rocks. I palpated the female and no additional offspring were detected inside her reproductive tract.

 On 12 October 2017, St09 was found alive in the north ditch of the dike road south of Pool 2 and north of Pool 5 and the inlet canal. The head was swollen, caked with blood, and the caudal approximately 1/5 of the body was cleanly severed and missing. This observation was during duck hunting season and in a high traffic area for hunters. I euthanized the individual according to IACUC protocol 16-0003.

Breeding and Courtship.– On 28 April 2017, St02 was observed in active copulation with a unmarked female. This pair was positioned in a linear fashion with heads opposite one another, in approximately 0.25-m *P. arundinacea* between the inlet canal north of Pool 5 and public dike road south of Pool 2 (Fig 2). This activity was approximately 2 m south of the public dike road.

Active courtship was observed between St13 and an unmarked male on 18 May 2017. These individuals were arranged in a crescent shape and the tail of the unmarked male was wrapped around the vent of the female (St13) and made jerky movements along her side and dorsum. The male would move his wrapped tail up and down the length of the tail of the female in asynchronous, rapid spurts of 4 to 8 repetitions. This encounter took place in dense *C. maculatum* atop dead *Bromus* sp.

Attendance, or a male positioned next to, on top of, or under a female in a stationary fashion during breeding season, was observed (3 radio tagged males and 3 radio tagged females) on several occasions (Table 5). Several of these individuals were observed in close proximity to one another on days adjacent to observed attendance behavior.

Maternal Attendance.– Maternal attendance was observed on 02 September 2016. During a scheduled tracking event, St03 was tracked to a small burrow (3-5 cm) in Pool 5 facing NNE and partially covered by *D. spicata*. The wire from the receiver was unhooked from the antenna and inserted into this burrow to confirm the location of St03. A neonate *S. tergeminus* was observed near the burrow entrance and shortly after, St03 was also observed at the entrance (Fig. 10). Another neonate joined them and both neonates showed clear evidence of being pre-ecdysis.

DISCUSSION

I hypothesized that *S. tergeminus* on the more natural Cheyenne Bottoms Preserve (CBP) owned by The Nature Conservancy (TNC) would have larger home range estimates than in the more constrained but apparently highly productive land on Cheyenne Bottoms Wildlife Area (CBWA) owned by Kansas Department of Wildlife, Parks and Tourism (KDWPT). I intended to track equal sex ratios on each property to assess differences in habitat use by sex within the two properties. Unfortunately a sufficient number of appropriately sized individuals could not be captured within the scope of this study. As such, a total of 3 snakes (2 male and 1 female) were implanted on the TNC property and a total of 15 snakes (10 males and 5 females) on the KDWPT property. The unequal, small sample size precluded a statistical test of this hypothesis. However, the two largest 100% MCP in this study were on the TNC property (St04 and St10 respectively, both males). The only female tracked on the TNC property (St06) had the second largest 100% MCP of all females (Table 1). This was also reflected in the mean home range sizes between the TNC property (100% MCP = 28.27 ha, 95% KDE = 2.07 ha) and KDWPT property (100% MCP = 9.05, 95% KDE = 0.29), albeit a largely uneven sample size.

 The mean home range estimates from 100% MCP were similar to those reported in Nebraska (Patten et al., 2016), but much smaller than those reported in Missouri (Durbian et al., 2008) (Table 6). The study sites in Nebraska and Missouri are wetlands superficially similar to Cheyenne Bottoms and one another. However, considering the constrained amount of land available in the KDWPT property, it is not as surprising that these home range estimates are smaller. The series of dike roads and pools created in the

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1950s for water management have provided a modified landscape that undoubtedly affects wildlife movements. When water levels are high, the amount of land available is reduced and the dike roads that transect the pools provide areas that seemingly concentrate high densities of snakes and other wildlife (Fig. 3). It was clear that these snakes, particularly males, make many and often large movements during the respective breeding seasons.

Three populations of *S. tergeminus* in Missouri (Durbian et al. 2008) had home range and core use areas larger than observed in this study or in Nebraska (Patten et al., 2016). The 2 study sites in Nebraska were relatively small (378 ha and 263 ha) and had small population estimates of *S. tergeminus*; 116 and 516 individuals, respectively (Patten et al., 2009; Patten et al., 2016). Both of these sites occurred in Pawnee County and analyses were combined. The 3 study sites in Missouri were larger (3,012 ha, 4,400 ha, and 1,443 ha) and more comparable in size to Cheyenne Bottoms (Durbian et al., 2008). It is counter intuitive that the home range and core use area estimates were more similar to the Nebraska population, however, this could be due to the highly altered landscape of the KDWPT property where the majority of individuals occurred. The home range estimates for several individuals were larger due to the inclusion of marginal habitats (Fig. 11). These MCP estimates on the KDWPT property included areas of open water that could not be used by this species. While the KDWPT property is highly altered, it is also has apparently high densities of potential prey items and a robust populations of *S. tergminus* (KBS, 1986; Collins et al., 2010).

 Bender (2009) reported small mammals were an important prey item in the diet of *S. tergeminus* at Cheyenne Bottoms, followed by snakes. Both of these groups were

observed and the Common Gartersnake (*Thamnophis sirtalis*) was frequently encountered throughout the year. Seigel (1986) documented *T. sirtalis* was prey of *S. tergeminus* in Missouri. The closely related Plains Gartersnake (*T. radix*) was also a common species in Cheyenne Bottoms.

On 26 August 2016 an unmarked *S. tergeminus*, that would later be implanted with a transmitter (St05), was observed just after envenomating a Brown-headed Cowbird (*Molothrus ater*), that was moving spasmodically on the ground next to the rattlesnake (Fig. 12). The predation event was observed from this time until the prey item was ingested, and was completed in approximately 24 minutes. Birds have been reported in the diet of *Sistrurus*, but constitute an insignificant portion of the diet and *M. ater* has never been reported as a prey source (Hallock, 1991; Patten et al., 2009). It remains unknown if this is a common occurrence or if this was an isolated event. Birds could be an important prey opportunity in Cheyenne Bottoms given both the large number of migrants and high density of breeding populations (Zimmerman, 1990; Skagen and Knopf, 1993; Penner, 2009). Rattlesnakes are known to prey on birds (Beavers, 1976; Clark, 2002; Dugan and Hayes, 2012). The Iranian spider-tailed viper (*Pseudocerastes urarachnoidesis*) has an elaborate caudal structure that it uses to lure birds within striking distance (Fathina et al., 2009; Fathina et al., 2015). The observation of 5 individuals, including St05, that spent time in cattails over standing water was surprising but given the seasonal abundance of avian prey, perhaps less so. It seems plausible that birds are consumed more frequently by *S. tergeminus* in this ecosystem than previously reported in the diet. The long duration spent in the cattails by St09 coincided with fledging of the Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*), Red-winged Blackbird

(*Agelaius phoeniceus*), and Sora (*Porzana carolina*) among others. These birds are common in Cheyenne Bottoms and are known to nest in cattails (Penner, 2009). If birds are being consumed by *S. tergeminus*, a number of young, clumsy fledging birds could provide a significant seasonal food source. This could help explain the high densities, small home ranges, and large overall size of *S. tergeminus* in Cheyenne Bottoms (Bender, 2009; Collins et al., 2010).

 Estimates for home range and core use area using 95% and 50% kernel density estimators (KDE) were calculated using least-squares cross validation (LSCV) (Durbian et al., 2008; Wastell and Mackessy, 2011; Patten et al., 2016). Concerns about the comparability of KDE using LSCV for herpetofaunal data were raised by Row and Blouin-Demers (2006). I report these values for consideration in the spatial ecology of *S. tergeminus*, and for consideration to the validity of comparing KDE using LSCV across herpetofaunal studies. Considering the small size of these estimates, I question whether they can provided useful estimates of home range and habitat use by snakes in this wetland.

 I provide 50% MCP estimates as a proxy for estimating core use area as a more repeatable estimate than 50% KDE (Johnson, 2000). The repeatable estimates from MCP of core use area that can be compared across studies. This method might not be as useful to study small scale habitat use as the method recommended by Row and Blouin-Demers (2006), but it is proposed as a metric useful to the conservation of these animals and to give insight into their ecology.

 The mean home range and core area use estimates for all estimators were larger in males than in females (Tables $2 \& 3$). Male home range estimates were larger than

females in Missouri, and in studies of *S. catenatus* (Johnson, 2000; Durbian et al., 2008; DeGregorio et al., 2011). During the spring of 2017, several males frequently made movements of 0.25 - 0.5 km. The end of frequent movements of this size coincided with the end of attendance of females by males and indicated the end of the spring breeding season. This suggest that the larger estimates seen in males could be caused by mate searching during the breeding season.

Females made fewer large movements than males. However in 2016, St03 moved long distances toward a hibernacula post parturition. Three of the 5 females gave birth during this study $(St03 - 2016, St13$ and $St14 - 2017)$, however samples sizes of females were too small to evaluate home ranges in females by reproductive status. Anecdotally, reproductive status of females did not produce a clear pattern in home range estimates. The largest female 100% MCP was that of a female during a season it gave birth (St03 - 2016). However, the second largest 100% MCP represented a female that did not give birth during the study (St06) but rather moved onto a cattail surrounded island on the TNC property. Two females (St13 and St15) on the KDWPT property spent the 2017 active season in close proximity to one another and made similar movements, though they differed in reproductive status.

Maternal attendance observed in this study by St03 in 2016 was consistent with reports of maternal attendance in *S. t. edwardsii* from Colorado (Wastell and Mackessy, 2016). In both accounts, the female was with her young while they were pre-ecdysis, and left soon after. This likely coinciding with the first shed of the neonates. In 2017, St13 was observed with 6 pre-ecdysis neonates. The neonates were observed within 1 m of St13 and retreated to the structure St13 was under. It was not possible to confirm if these

were her offspring and St13 did not actively become visible when the neonates fled to cover.

 The large number of mortalities in this study was not expected. Six of the 18 individuals (33%) died prior to the completion of this study (Table 4). This mortality rate is higher than the active season mortality rate (21%) reported by Harvey and Weatherhead (2006) for *S. catenatus* in Ontario, Canada. Mortalities due to humanrattlesnake interactions were expected. I suspect humans caused only 1 mortality in this study. Five of the 18 adult individuals died of presumably natural predation events, as the individuals were known to be healthy just prior to death. The large number of wading birds, raptors, *L. holbrooki*, and mustelids present a substantial threat to snake species in Cheyenne Bottoms. Individuals being struck by motor vehicles is also a common source of mortality in Cheyenne Bottoms.

 Home range and core area estimates for *S. tergeminus* varied in this study. Males had larger values across all home range and core use area measures. As reported elsewhere, crayfish burrows were used as hibernacula in Cheyenne Bottoms (Patten et al., 2016). Here I report the first observation of maternal attendance by *S. t. tergeminus* and the first documentation of *M. ater* in the diet of *S. tergeminus*.

The information reported here gives further insight into the biology of *S. tergeminus* and is important to the conservation of this species in Kansas and across its range in the central United States and northern Mexico (Powell et al., 2016). It is currently listed as Threatened in Nebraska and Endangered in Missouri, and might not be as secure in Kansas as once thought (Bender, 2009; MDC, 2018; NGPC, 2018). Considering the confirmation that crayfish burrows are important to the natural history of

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Table 1. Home range estimates with 100% minimum convex polygons (MCP) and 95% Fixed-kernel density estimates (FK) and core area estimates with 50% MCP and 50% FK in hectares for *Sistrurus tergeminus* in Cheyenne Bottoms, Barton County, Kansas during 2016 and 2017.

Individual	Sex	100% MCP	50% MCP	95% FK	50% FK	Locations
St01	Male	11.562	0.713	0.529	0.014	68
StO2	Male	13.377	0.984	4.13	0.053	41
StO3	Female	22.003	6.576	1.661	0.209	50
St ₀₄	Male	60.992	13.479	5.121	0.16	74
St ₀₆	Female	5.846	3.687	0.266	0.009	49
St ₀₇	Male	13.535	0.351	0.625	0.028	50
St ₀₈	Male	9.621	0.278	2.104	0.106	51
St ₀₉	Male	17.867	3.132	2.16	0.082	40
St10	Male	17.96	4.744	0.829	0.017	46
St11	Male	10.616	4.666	2.571	0.137	48
St12	Male	4.058	1.52	0.401	0.004	48
St13	Female	0.462	0.046	0.225	0.007	48
St15	Female	0.374	0.057	0.12	0.004	46
St16	Male	0.888	0.099	0.066	0.001	44
St17	Female	4.292	1.669	0.285	0.012	41

Table 2. Home range estimates with 100% minimum convex polygons (MCP) and 95% Fixed-kernel density estimates (FK) and core area estimates with 50% MCP and 50% FK in hectares for male *Sistrurus tergeminus* with 30 or more unique locations in Cheyenne Bottoms, Barton County, Kansas during 2016 and 2017.

Individual	100% MCP	50% MCP	95% FK	50% KF	Locations
St01	11.562	0.713	0.529	0.014	68
St ₀₂	13.377	0.984	4.13	0.053	41
St ₀₄	60.992	13.479	5.121	0.16	74
St ₀₇	13.535	0.351	0.625	0.028	50
St ₀₈	9.621	0.278	2.104	0.106	51
St ₀₉	17.867	3.132	2.16	0.082	40
St10	17.96	4.744	0.829	0.017	46
St11	10.616	4.666	2.571	0.137	48
St12	4.058	1.52	0.401	0.004	48
St16	0.888	0.099	0.066	0.001	44

Table 3. Home range estimates with 100% minimum convex polygons (MCP) and 95% Fixed-kernel density estimates (FK) and core area estimates with 50% MCP and 50% FK in hectares for female *Sistrurus tergeminus* with 30 or more unique locations in Cheyenne Bottoms, Barton County, Kansas during 2016 and 2017.

Individual 95% FK 50% FK 100% MCP 50% MCP Locations 6.576 22.003 0.209 50 1.661				
	St03			
St ₀₆ 3.687 0.009 5.846 49 0.266				
St13 48 0.462 0.046 0.007 0.225				
St15 0.374 0.057 0.004 46 0.12				
St17 4.292 1.669 0.012 0.285 41				

Individual	Date Discovered Dead	Cause of Death	Previous Injury Noted	Assumed Predator	Predator Confirmed	Condition of Carcass
St01	04 September 2017	Unknown	N ₀	Unknown	N _o	Decayed
St02	09 May 2017	Predation	N ₀	Unknown	N ₀	Only Transmitter
St03	15 June 2017	Previous Injury	Yes	Unknown	N ₀	Fresh - Whole
St05	14 October 2016	Predation	N ₀	Small Carnivore	N ₀	Only Transmitter
St09	12 October 2017	Euthanasia	No	Human	N ₀	Mostly Whole
St14	17 August 2017	Predation	N ₀	Speckled Kingsnake (Lampropeltis holbrooki)	Yes	Fresh - Whole

Tables 4. Information on the deaths and predation of radio tagged *Sistrurus tergeminus* during the 2016 and 2017 field seasons.

Table 6. Home range estimates using 100% minimum convex polygon (MCP) and 95% kernel density estimates (FK) and core area estimates using 50% FK in hectares from 4 study sites used for comparison to this study.

Fig 1. Range of *Sistrurus tergeminus* (Western Massasauga) in the United States and northern Mexico. The Prairie Massasauga (*S. t. tergeminus*) is pictured in green and the Desert Massasauga (*S. t. edwardsii*) is pictured in rose. Map adapted from Powell et al. 2016.

Fig 2. Site map of Cheyenne Bottoms in Barton County, Kansas. The 8,036 ha of the Cheyenne Bottoms Wildlife Area owned by Kansas Department of Wildlife, Parks, and Tourism (KDWPT) is in green and the 3,114 ha of the Cheyenne Bottoms Preserve owned by The Nature Conservancy (TNC) is in tan. The pools listed (Pool $1 - 5$) designate areas of water controlled by KDWPT. Pool 1 consistently has standing water and is not able to be used by *S. tergeminus*. Other pools vary in water level.

Fig 3. Aerial imagery (National Agriculture Image Program (NAIP) – 2015) of Cheyenne Bottoms in Barton County, Kansas. The 8,036 ha of the Cheyenne Bottoms Wildlife Area owned by Kansas Department of Wildlife, Parks, and Tourism (KDWPT) is in outlined green and the 3,114 ha of the Cheyenne Bottoms Preserve owned by The Nature Conservancy (TNC) is outlined in tan. Points of individual locations, 100% minimum convex polygon (MCP) and 50% MCP are shown for 15 *Sistrurus. tergeminus* with 30 or more locations in 2016-2017. Note the large amounts of surface water.

Fig. 4. Radiograph of a *Sistrurus tergeminus* (St05) showing a 9g Holohil SI-2 transmitter (Holohil Systems Ltd., Ontario, Canada) in the coelomic cavity with the antenna running cranial of the transmitter subcutaneously.

Fig. 5. Estimations of home range and core use area of *Sistrurus tergeminus* using 100% minimum convex polygon (MCP) and 50% MCP respectively, in 2016 and 2017. This is for the 3 individuals (St04, St06, and St10) on the Cheyenne Bottoms Preserve owned by The Nature Conservancy in Barton County, Kansas. Two of these are male (St04 and St10) and one is female (St06).

Fig. 6. Estimations of home range and core use area of *Sistrurus tergeminus* using 100% minimum convex polygon (MCP) and 50% MCP respectively, in 2016 and 2017. This is for the 15 individuals with 30 or more unique locations analyzed during this study. The Cheyenne Bottoms Wildlife Area owned by the Kansas Department of Wildlife, Parks and Tourism is represented in the green and the Cheyenne Bottoms Preserve owned by The Nature Conservancy is represented in tan. Both properties are located in Barton County, Kansas.

Fig. 7. Estimations of home range and core use area of *Sistrurus tergeminus* using 95% fixed-kernel density estimates (FK) and 50% FK respectively using least-squares cross validation to select a smoothing factor. This is for 3 individuals (St04, St06, and St10) on the Cheyenne Bottoms Preserve owned by The Nature Conservancy in Barton County, Kansas.

Fig. 8. Estimations of home range and core use area of *Sistrurus tergeminus* using 95% kernel density estimators (KDE) and 50% KDE respectively, in 2016 and 2017. This is for the 15 individuals with 30 or more unique locations analyzed during this study. The Cheyenne Bottoms Wildlife Area owned by the Kansas Department of Wildlife, Parks and Tourism is represented in the green and the Cheyenne Bottoms Preserve owned by The Nature Conservancy is represented in tan. Both properties are located in Barton County, Kansas.

Fig. 9. Aerial image (National Agriculture Imagery Program (NAIP) - 2015) showing the locations, 100% minimum convex polygon (MCP) outlined in yellow and 50% MCP outlined in orange for a *Sistrurus tergeminus* (St09) in Cheyenne Bottoms in Barton County, Kansas. Vegetation pictured here was primarily emergent *Typha* sp. in 2017 when this data was collect, open water existed near the dike road to the north. The area north of the dike road pictured at the bottoms is Pool 2 of the Cheyenne Bottoms Wildlife Area owned by the Kansas Department of Wildlife, Parks and Tourism. St09 spent 26 consecutive tracking events over 13 weeks in these cattails above or in standing water.

Fig. 10. Maternal attendance observed on 02 September 2016. St03 was tracked to a small burrow (3-5 cm) in Pool 5 facing NNE and slightly covered by *D. spicata* where 2 pre-ecdysis neonates were observed in this burrow with St03.

Fig. 11. Aerial image (National Agriculture Imagery Program (NAIP) - 2015) showing the locations, 100% minimum convex polygon (MCP) outlined in yellow and 50% MCP outlined in orange for a *Sistrurus tergeminus* (St08) in Cheyenne Bottoms in Barton County, Kansas. . This depicts inclusion of marginal habitat (open water) in a 100% MCP. Vegetation pictured here is was mostly emergent *Typha* sp. in 2017 when this data was collect, open water existed on both sides of the dike road depicted in the center where all locations (mustard) are present. The area northwest of the dike road pictured at Cheyenne Bottoms is Pool 2 and the area southeast is Pool 1 of the Cheyenne Bottoms Wildlife Area owned by the Kansas Department of Wildlife, Parks and Tourism.

Fig. 12. Predation event on 26 August 2016 when an unmarked *Sistrurus tergeminus* that would later be implanted (St05) was observed consuming a Brown-headed Cowbird (*Molothrus ater*). This observation started post envenomation but while the *M. ater* was alive (Top), then consumption continued (Bottom Left), until ingestion was complete and a large bolus was noted (Bottom Right).

Appendix 1. *Sistrurus tergeminus* implanted with very high frequency (VHF) transmitters in Cheyenne Bottoms in Barton County, Kansas during 2016-2017. These individuals were captured on the Cheyenne Bottoms Wildlife Area (CBWA) owned by the Kansas Depart or the Cheyenne Bottoms Preserve (CBP) owned by The Nature Conservancy. An * denotes the last day a signal was found for individuals whose signal was lost and never recovered.

Individual	Sex	Capture Date	Property	Final Tracking Day	Reason to	2016	2017
					end Tracking	Locations	Locations
St01	Male	16 July 2016	CBWA	04 September 2017	Death	30	38
St ₀₂	Male	17 July 2016	CBWA	09 May 2017	Death	31	10
StO3	Female	19 July 2016	CBWA	15 June 2017	Death	30	20
St ₀₄	Male	08 August 2016	CBP	26 October 2017	End of Study	20	54
St ₀₅	Male	25 August 2016	CBWA	14 October 2016	Death	11	N/A
St06	Female	09 April 2017	CBP	26 October 2017	End of Study	N/A	49
St ₀₇	Male	14 April 2017	CBWA	26 October 2017	End of Study	N/A	50
St ₀₈	Male	15 April 2017	CBWA	26 October 2017	End of Study	N/A	51
St ₀₉	Male	15 April 2017	CBWA	12 October 2017	Euthanized	N/A	40
St10	Male	18 April 2017	CBP	26 October 2017	End of Study	N/A	46
St11	Male	20 April 2017	CBWA	26 October 2017	End of Study	N/A	48
St12	Male	24 April 2017	CBWA	26 October 2017	End of Study	N/A	48
St13	Female	24 April 2017	CBWA	26 October 2017	End of Study	N/A	48
St14	Female	28 April 2017	CBWA	27 August 2017	Death	N/A	28
St15	Female	05 May 2017	CBWA	26 October 2017	End of Study	N/A	46
St16	Male	13 May 2017	CBWA	26 October 2017	End of Study	N/A	44
St17	Female	18 May 2017	CBWA	26 October 2017	End of Study	N/A	41
St18	Male	29 May 2017	CBWA	10 July 2017*	Lost Signal	N/A	10